Atty. Dkt. No. 038602-1260

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date: 5/8/02

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## VERSION WITH MARKINGS TO SHOW CHANGES MADE

Marked up replacement paragraphs:

**Page 9**, delete the 4th, 5th and 6th paragraphs:

Figure 9. Shared amino acid sequence homology of MKK1 SEQ ID NO: 2 and csk SEQ ID NO: 7.

Figures 10A and 10B SEQ ID NOS 4, 8-10 respectively, in order of appearance. Shared amino acid sequence homology of MKK2 and atk/btk.

Figures 11A, 11B, 11C and 11D SEQ ID NOS 6, 11-19, respectively, in order of appearance.

**Page 13**, delete the 3rd and 4th paragraphs:

The nucleotide and deduced amino acid sequence of human MKK1, MKK2, and MKK3 are shown in Figures 1A-1B (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) and 3A-3B (SEQ ID NOS 5-6), respectively. Figures 9 (SEQ ID NOS 2 and 7, respectively, in order of appearance), 10A-10B (SEQ ID NOS 4, 8-10, respectively, in order of appearance) and 11A-11D (SEQ ID NOS 6, 11-19, respectively, in order of appearance) show the shared sequence homology between MKKs and related tyrosine kinases.

**5.1 The MKK Coding Sequences**

The nucleotide coding sequence and deduced amino acid sequence of the human MKK1, MKK2, and MKK3 genes are depicted in Figures 1A-1B (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) and 3A-3B (SEQ ID NOS 5-6), respectively. In accordance with the invention, any nucleotide sequence which encodes the amino acid sequence of an MKK gene product can be used to generate recombinant molecules which direct the expression of an MKK.

**Pages 13-14**, delete the last paragraph:

In a specific embodiment described herein, the human MKK1, MKK2, and MKK3 genes were isolated by performing polymerase chain reactions (PCR) in combination

with two degenerate oligonucleotide primer pools that were designed on the basis of highly conserved sequences within the kinase domain of receptor tyrosine kinases corresponding to the amino acid sequence HRDLAA (residues 350-355 of SEQ ID NO: 2) (sense primer) and [SDVWS/FY] SDVWSF/Y (SEQ ID NO:24) (antisense primer) (Hanks *et al.*, 1988). The MKK cDNAs were synthesized by reverse transcription of poly-A RNA from the human K-562 cell line, ATCC accession number CCL 243, or from the Meg 01 cell line, (Ogura *et al.,* Blood 66: 1384 (1985)).

**Page 14**, delete 1st paragraph:

The PCR fragments were used to screen a lambda gt11 library of human fetal brain. For each individual MKK, several overlapping clones were identified. The composite of the cDNA clones for MKK1, MKK2, and MKK3 are depicted in Figures 1A-1B (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) , and 3A-3B (SEQ ID NOS 5-6), respectively.

**Pages 37-38**, delete the last paragraph:

cDNA was used in a polymerase chain reaction under standard conditions (*PCR Technology-Principles and Applications for DNA Amplifications*, H.E. Erlich, Ed., Stockton Press, New York 1989). Degenerate pools of primers corresponding to the amino acid sequence HRDLAA (residues 350-355 of SEQ ID NO:2) and SDVWSF/Y (SEQ ID NO:24) were prepared and used for the amplification:

5′ oligo pool

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                H   R   D   L   A   A
5′ GGAATTCC CAC AGN GAC TTN GCN GCN AG 3′ (SEQ ID NO: 20)
               T C   A  T C   A   A  C
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3′ oligo pool

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              F/Y   S   W   V   D   S
5′ GGAATTCC GAA NGT CCA NAC GTC NGA 3′ (SEQ ID NO: 21)
            ATG  CA         C    C
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Thirty-five PCR cycles were carried out using 8 μg (0.8 μg) of the pooled primers. (Annealing 55°C, 1 min; Extension 72°C, 2 min; Denaturation 94°C, 1 min). The reaction product was subjected to polyacrylamide gelelectrophoresis. Fragments of the

expected size (~210 bp) were isolated, digested with the restriction enzyme EcoRI, and subcloned into the pBluskript vector (Stratagene) using standard techniques (*Current Protocols in Molecular Biology*, eds. F.M. Ausubel *et al.*, John Wiley & Sons, New York, 1988).

**Page 38**, delete the last paragraph:

The partial cDNA sequence of the new MKK1 TK, which was identified by PCR, was used to screen a λgt11 library from human fetal brain cDNA (Clontech)(complexity of $1x10^{10}$ recombinant phages). One million independent phage clones were plated and transferred to nitrocellulose filters following standard procedures (Sambrook, H.J., Molecular Cloning, Cold Spring Harbor Laboratory Press, USA, 1989). The filters were hybridized to the EcoRI/EcoRI fragment of clone MKK1, which had been radioactively labeled using 50μCi [$\alpha^{32}$P]ATP and the random-primed DNA labeling kit (Boehringer Mannheim). The longest cDNA insert of ~3500 bp was digested with the restriction enzymes EcoRI/SacI to obtain a 5′ end probe of 250 bp. This probe was used to rescreen the human fetal brain library and several overlapping clones were isolated. The composite of the cDNA clones of MKK1, MKK2 and MKK3 is shown in Figures 1A-1B (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) and 3A-3B (SEQ ID NOS 5-6), respectively. The 1.75 million independent phage clones of a human placenta library, λZAP, were plated and screened with the 5′ end probe (EcoRI/SacI) of the clone used above. Subcloning of positive bacteriophages clones into pBluskript vector was done by the *in vivo* excision protocol (Stratagene).

**Page 39**, delete the 2nd paragraph:

The composite cDNA sequence and the predicted amino acid sequence of MKK1, MKK2 and MKK3 are shown in Figures 1A-1B (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) and 3A-3B (SEQ ID NOS 5-6), respectively.

**Page 41**, delete the 1st paragraph:

**8. Example: Production of Anti-MKK Antibodies and Immunoprecipitation of MKK Antibodies and Immunoprecipitation of MKK**

Antibodies recognizing MKK1 and MKK2 protein were made in rabbits using standard procedures. The anti-carboxy terminus MKK1 antibody was generated using the synthetic peptide GQDADGSTSPRSQEP (SEQ ID NO 22). The amino-terminus MKK1 Ab was generated using a GST-fusion proteins containing 78 amino acids coded by the SmaI to BG12 fragment of the MKK1 gene. The anti-carboxy terminus MKK2 Ab was made using a synthetic peptide corresponding to the sequence QQLLSSIEPLREKDKH (SEQ ID NO 23).

Marked up rewritten claims:

15. (Amended) The isolated recombinant MKK1 of Claim 14 comprising the amino acid sequence depicted in Figures 1A and 1B (SEQ ID NOS 1-2).

17. (Amended) The isolated recombinant MKK2 of Claim 16 comprising the amino acid sequence depicted in Figures 2A and 2B (SEQ ID NOS 3-4).

19. (Amended) The isolated recombinant MKK3 of Claim 18 comprising the amino acid sequence depicted in Figures 3A and 3B (SEQ ID NOS 5-6).